



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Gut microbiota-dependent anti-obesity mechanisms of Pu-erh tea polysaccharides: a comparative study of raw and ripened forms

Jing Ye^{a,b*}, Weipeng Pan^a, Yucheng Yang^{a,b}, Na Zhang^{a,b}, Bingde Zheng^{a,b}, Hongjie Yang^{a,b},
Pengfei Tu^c, Meitian Xiao^{a,b}

^aCollege of Chemical Engineering, Huaqiao University, Xiamen 361021, China

^bXiamen Engineering and Technological Research Center for Comprehensive Utilization of Marine Biological Resources, Xiamen 361021, China

^cSchool of Pharmaceutical Sciences, Peking University, Beijing 100191, China

ABSTRACT: Pu-erh tea, a well-known fermented tea, is classified into raw and ripened types, both associated with weight loss. As key components, raw Pu-erh tea polysaccharides (R-TPS) and ripened Pu-erh tea polysaccharides (F-TPS) are known to regulate blood glucose and lipids, contributing to anti-obesity effects. However, differences in their mechanisms remain unclear, limiting their targeted development. This study characterized their structures and evaluated their effects in high-fat diet-induced obese mice using 16S rRNA sequencing and fecal microbiota transplantation (FMT). Compared to R-TPS, F-TPS contained more galactose, arabinose, rhamnose, and glucose but had a higher molecular weight, while R-TPS was richer in galacturonic acid. Both polysaccharides alleviated weight gain and reshaped gut microbiota. Notably, F-TPS more effectively improved glucose homeostasis, enhanced lipolysis, and reduced inflammation, whereas R-TPS primarily inhibited adipogenesis. Crucially, these differential effects were characterized by distinct gut microbiota-dependent associations: F-TPS enrichment of Muribaculaceae, Lachnospiraceae, and Lactobacillaceae was closely correlated with improved glucose metabolism, lipid metabolism, and inflammation, while R-TPS specifically increased Akkermansiaceae abundance, which correlated with inhibited adipogenesis and utilization of energy. These findings underscore the potential of R-TPS and F-TPS as functional foods for personalized obesity management via gut microbiota modulation.

Keywords: Raw and ripened Pu-erh tea, polysaccharides, obesity, gut microbiot

1. Introduction

Obesity has become a growing public health problem worldwide. According to the latest statistics from the World Health Organization (WHO), in 2022, 43% of adults aged 18 years and older were overweight, and 16% were living with obesity.^[1] Obesity not only affects an individual's appearance and quality of life, but is also closely related to the occurrence of various chronic diseases, such as diabetes, cardiovascular disease, hypertension, etc., which brings a heavy medical burden to both individuals and the society.^[2-4] Therefore, the

*Corresponding author
yejenny@hqu.edu.cn

Received 28 August 2025
Received in revised form 29 December 2025
Accepted 19 March 2026

pursuit of safe and effective weight loss approaches has become a current research hotspot in the field of medicine and nutrition.

Among various anti-obesity strategies, dietary intervention remains a promising and practical approach. The traditional beverage Pu-erh tea has gained wild attention for its widely recognized weight loss benefits.^[5] Pu-erh tea is originated from Yunnan Province, China and has a long history of consumption. It is categorized into two major types-raw (Sheng) and ripened (Shu)-which differ significantly in terms of processing methods, chemical composition, and sensory characteristics.^[7] Interestingly, both raw and ripened Pu-erh tea have been reported to be associated with weight loss, and both have their own characteristics in terms of their weight loss effects.^[8] Studies have reported that raw Pu-erh tea is rich in tea polyphenols, which have the function of inhibiting fat synthesis and promoting fat decomposition, and can help reduce body weight to a certain extent.^[8, 9] While ripened Pu-erh tea has been fermented for a long period of time during the manufacture process, with the chemical compositions has been profoundly changed, producing substances such as theaflavin, which has the effect of regulating blood lipids and reducing fat synthesis.^[10] Our previous study has shown that raw Pu-erh tea and ripened Pu-erh tea extracts have similar effects on reducing body weight and fat accumulation in obese mice, but the two extracts exert significant differences in regulating intestinal flora.^[11] Some studies have shown that raw Pu-erh tea polysaccharides (R-TPS) and ripened Pu-erh tea polysaccharides (F-TPS) have the effect of regulating blood glucose and blood lipids, which may be related to the weight loss effect^[12]. However, systematic comparative studies focusing on the weight-reducing effects of R-TPS and F-TPS remain scarce, and the specific roles and differences of these polysaccharides in modulating host metabolism and gut microbiota are still poorly understood. We hypothesize that although R-TPS and F-TPS may both exert anti-obesity effects, they do so via distinct pathways, leading to differences in their mechanisms of action and metabolic outcomes.

In the present study, the effects of R-TPS and F-TPS on obesity in HFD-fed mice were systematically evaluated to explore the similarities and differences in their weight loss effects. The effects of R-TPS and F-TPS on the composition and structure of the gut microbiota were also examined. In addition, fecal microbiota transplant (FMT) experiments were performed to verify whether the anti-obesity effects of R-TPS and F-TPS were dependent on alterations in the gut microbiota. This study will provide new insights to understand the potential anti-obesity effects and mechanisms of R-TPS and F-TPS, as well as provide a basis for elucidating the similarities and differences in their ameliorative effects on obesity.

2. Materials and methods

2.1 Preparation of R-TPS and F-TPS

The raw and ripened Pu-erh tea were obtained from Tianxing Pu-erh Tea Co., Ltd, Pu er, China, in 2020. The preparation of Pu-er tea polysaccharides was as follows: 100 g raw/ripened Pu-erh tea was soaked in 95% ethanol for 24 h, the residue was extracted by reflux with 2 L of water in a water bath at 80 °C for 2 h, then filtered, and repeated extraction of filter residue for 2 times. The extracts were then combined and concentrated to 400 mL by rotary evaporation. The concentrated solution was centrifuged at 5000 r/min for 10 min to

remove small particles. Then, the supernatant was added with 95% ethanol to reach the final concentration of 80%, and the precipitation was crude polysaccharide, which was subsequently experienced deproteinization process by Sevag method and lyophilization to obtain raw and ripened Pu-erh tea polysaccharide. The resulting polysaccharides were further purified by anion-exchange chromatography on a DEAE-52 cellulose column, and sequentially eluted with distilled water and 2.0 mol/L NaCl solution. The eluates from both elution steps were collected based on the phenol-sulfuric acid detection, combined, dialyzed (cutoff Mw: 3500 Da) against distilled water, and lyophilized to obtain the final R-TPS and F-TPS. The chemical compositions of R-TPS and F-TPS were analyzed as the reported methods with little modifications. The content of total polysaccharides in R-TPS and F-TPS was measured colorimetrically by anthrone-sulfuric acid colorimetry with little modification. The analysis of monosaccharide compositions was performed on an Agilent 1260 Infinity II HPLC (Agilent Technologies, Germany) with a ShimNex CS C18 (5 μ m, 4.6 mm $\times$ 250 mm, Shimadzu, Tokyo, Japan).

2.2 Animal and diet

50 male C57BL/6 mice at 6 weeks of age with body weight of (16 ± 0.85) g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (SCXK (Shanghai) 2017-0005, Shanghai, China). All the animal experiments were allowed and performed according to the NIH (National Research Council) Guide for the Care and Use of Laboratory Animals and the Guidelines of the Laboratory Animal Ethical Committee of Huaqiao University (A2024071). The experimental mice were raised in the controlled environment with the temperature kept at (20 ± 2) °C, the humidity was set at 40–60 %, and a light and dark cycle of 12 h in specific pathogen free lab. After acclimatization for a week, the mice were randomly divided into five groups (n = 10 per group): normal-chow diet group (10 kcal% from fat, Research Diets TP23302) with 200 μ L saline as the normal control (ND), high fat diet group (60 kcal% from fat, Research Diets TP23300) with 200 μ L saline as the model control (HFD), positive control group (AL) having HFD with treatment of 60 mg/kg/d orlistat, R-TPS and F-TPS groups having HFD with administration of 300 mg/kg/d R-TPS and F-TPS, respectively. The dose of 300 mg/kg/d was selected based on previous literature demonstrating the efficacy and safety of tea polysaccharides in murine models [12, 15], as well as our preliminary screening experiments showing effective metabolic improvements at this concentration. All the treatments were given by intragastric gavage and lasted for 14 weeks. During the period of experiment, all mice had *ad libitum* access to diet and water and the body mass and consumption of food were recorded weekly. At the end of the experiment, the mice were euthanized after 12 h of fasting. The serum, liver, perirenal and epididymal adipose tissues and feces were collected and stored at -80 °C for further experiments.

The FMT was conducted according to the methodology previously described in the literature^[13], where the C57BL/6 mice were given antibiotics (neomycin sulfate, ampicillin, metronidazole, and vancomycin with dosages of 200, 200, 200, and 100 mg/kg) by oral gavage once each day for two weeks to deplete the gut microbiota (recipient mice). Mouse feces were gathered in the F-TPS and R-TPS interference assay and carefully homogenized in the phosphate buffered saline (PBS) solution to a concentration of 100 mg/mL,

which then was centrifuged at 800 g for 5 min. Subsequently, the obtained supernatant was centrifuged at 4000 g for 15 min, the precipitation was then harvested to re-suspended in PBS to obtain the fecal microbial solution. The recipient mice were intragastrical administered with 200 μ L of the microbial solution freshly prepared from feces of corresponding donor mice every day. The FMT experiment lasted for 14 weeks and mice were fed HFD during the transplantation, then, the liver, adipose tissues, and cecum contents were harvested and preserved at - 80 °C for further analysis.

2.3 Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

OGTT was performed according to the previous method.^[14] Briefly, the mice were fasted for more than 8 h and subsequently followed by weighting body mass and oral treatment of 2 g/kg glucose by gavage. Then, the content of blood glucose was detected at 0, 30, 60, 90, and 120 min. ITT was conducted three days after OGTT. Given insulin (0.35 U/kg) by intraperitoneal injection, the mice were treated blood sampling every 30 min up to 2 h to detect insulin contents. The glucose concentration-time graph was then drawn to calculate the integrated areas under each curve (AUC). Subsequently, homeostasis model assessment-insulin resistance (HOMAIR) was calculated as follows: $\text{HOMAIR} = \text{fasting insulin (mU/L)} \times \text{fasting glucose (mmol/L)} / 22.5$.

2.4 Biochemical analysis

The contents of serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), and acid phosphatase (ACP) were determined by the commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentrations of serum tumor necrosis factor α (TNF- α), lipopolysaccharide (LPS), C-reactive protein (CRP), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) were tested by the commercial enzyme-linked immunosorbent assay (ELISA) kit (Proteintech China, Wuhan, China).

2.5 Histology analysis

Freshly harvested mice liver, epididymal and perirenal fat tissues were fixed in 4% paraformaldehyde overnight, followed by gradient dehydration and embeddedness in paraffin. Then the blocks were cut into slices section of 6 μ m and processed hematoxylin and eosin (H&E) staining for morphological observation.

2.6 RNA preparation and Quantitative real-time PCR (q-PCR) analysis

The q-PCR analysis was conducted as the previous method.^[11] Briefly, hepatic tissues were homogenized, and total RNA was obtained using Spin Column Animal Total RNA Purification Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Subsequently, reverse transcription of the extracted RNA was conducted by BeyoRTTMM-MuL V transcriptase kit (Beyotime, Shanghai, China), and q-PCR was performed with SG Fast qPCR Master Mix (2X) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) on Bio-Rad CFX96 (Bio-Rad Laboratories, Inc) following the protocols of manufacture. Samples were analyzed in triplicate, and the levels of the target genes were calculated by $2^{-\Delta\Delta C_t}$ method and GAPDH was the endogenous reference. The analyzed target genes were glucose transporter 4 (Glut4), insulin receptor

substrate 1 (IRS1), carnitine palmitoyl transferase-1 (CPT-1), peroxisome proliferator-activated receptor γ (PPAR γ), peroxisome proliferator-activated receptor α (PPAR α), sterol regulatory element-binding protein 1c (SREBP-1c), liver X receptor (LXR α), fatty acid synthase (FAS), etc. The primer sequences shown in Table S1 were gained from Sangon Biotech (Sangon Biotech Co., Ltd. Shanghai. China).

2.7 Western blotting analysis

Western blotting analysis was performed using our previous method^[11]. Briefly, the hepatic tissues were homogenized and lysed in radio immunoprecipitation assay (RIPA) buffer (Sigma, St. Louis, MO, USA) followed by centrifuging at 4 °C, 10,000 g for 20 min and the supernatant was collected. The protein content of the supernatant was measured using BCA protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instruction. Equal amount of protein (20 μ g/lane) was separated by 10% SDS-PAGE and blotted onto a polyvinylidene difluoride (PVDF) membrane. After sealed with 5% (w/v) skimmed milk in TBST, the membranes were then incubated with primary antibodies overnight at 4 °C followed by incubating in horseradish peroxidase-conjugated secondary antibody for 1.5 h. Finally, the proteins levels were detected by enhanced chemiluminescence (ECL). GAPDH was used as internal control.

2.8 Short chain fatty acids (SCFAs) content analysis

The measurement of fecal SCFAs content was executed following the previous method with slight modification.^[2] Briefly, the feces samples were soaked in 1 mL phosphoric acid aqueous solution (0.5%, v/v) on ice followed by centrifugation at 13,000 r/min for 10 minutes, the obtained supernatant was extracted for SCFAs using ethyl acetate (EA), the EA supernatant was then filtered with a 0.22 μ m membrane for further analysis.

The SCFAs contents were measured on an Agilent 7890N gas chromatography (Agilent, USA) equipped with an FID detector and a DB-WAX column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). The injection volume was 1 μ L with the split ratio of 1:10. The working temperature of the injector and detector was both set at 250 °C. The initial column temperature was set at 85 °C and kept for 3 min, then heated up to 170 °C with the heating rate of 10 °C/min, and held at 170 °C for 12 min.

2.9 Gut microbiota analysis

Total DNA of cecum contents was extracted and 16S rRNA genes of V3-V4 regions were amplified. The sequencing libraries were prepared and sequenced using Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) based on the Illumina Mi Seq platform. Sequences with $\geq$ 97% similarity were classified into identical operational taxonomic units (OTUs) according to Ribosomal Database Project (RDP) by UPARSE.

The community richness indices of Alpha diversity including Chao1, ACE, Shannon, Simpson. Beta diversity was analyzed with QIIME (Version 1.7.0). Principal Coordinate Analysis (PCoA) was conducted to get principal coordinates and visualize from complex, multidimensional data. LEfSe software was used for LEfSe analysis, and the default LDA score was set at 4. Spearman analysis was performed to test the

significance of microbiota species and environmental factors, which was then visualized by heatmap function in the heatmap package.

2.10 Statistical analysis

All data in this study were presented as the mean $\pm$ standard deviation ($X \pm SD$). The statistical significances of the data between each group were evaluated by a one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test for multiple comparisons using SPSS 22.0 (IBM, U.S.A.).

3. Results

3.1 Structural characterization of F-TPS and R-TPS

3.1.1 Chemical characterization of F-TPS and R-TPS

The chemical analyses were conducted to characterize the physicochemical properties of F-TPS and R-TPS. As shown in Table 1, F-TPS contained higher levels of total sugar, uronic acid, and protein compared to R-TPS, while polyphenols content was higher in R-TPS than that in F-TPS. The monosaccharide composition of R-TPS primarily consisted of GalA, Gal, and Ara, with small molar amounts of Man, Glc, Rha, and Rib, in a molar ratio of 1:0.5:0.5:0.1:0.2:0.1:0.06. In contrast, F-TPS was mainly composed of Gal, GalA, Ara, Rha, Man, Glc, and GlcA, with a molar ratio of 1:0.9:0.8:0.7:0.4:0.3:0.1. The results of HPGPC indicated that both R-TPS and F-TPS were heterogeneous polysaccharides consisted of three peaks (Fig. S1a). R-TPS was composed of molecular weight of 291.76 kDa, 184.89 kDa, and 170.15 kDa, with percentage of 52.43%, 39.31%, and 8.26%, respectively. While F-TPS was composed of molecular weight of 623.52 kDa, 402.56 kDa, and 77.59 kDa, with percentage of 50.85%, 42.50%, and 6.65%, respectively.

Table 1 The physicochemical properties of F-TPS and R-TPS

	R-TPS	F-TPS
Total sugar (%)	44.4 $\pm$ 0.087	48.5 $\pm$ 0.078
Uronic acid (%)	31.4 $\pm$ 0.071	34.2 $\pm$ 0.082
Protein (%)	2.66 $\pm$ 0.014	9.86 $\pm$ 0.056
Polyphenol (%)	5.57 $\pm$ 0.024	3.70 $\pm$ 0.052
Monosaccharide composition (mol%)	GalA:Gal:Ara:Man:Glc:Rha:Rib (1:0.5:0.5:0.1:0.2:0.1:0.06)	Gal:GalA:Ara:Rha:Man:Glc:GlcA (1:0.9:0.8:0.7:0.4:0.3:0.1)

3.1.2 UV and FT-IR analysis of F-TPS and R-TPS

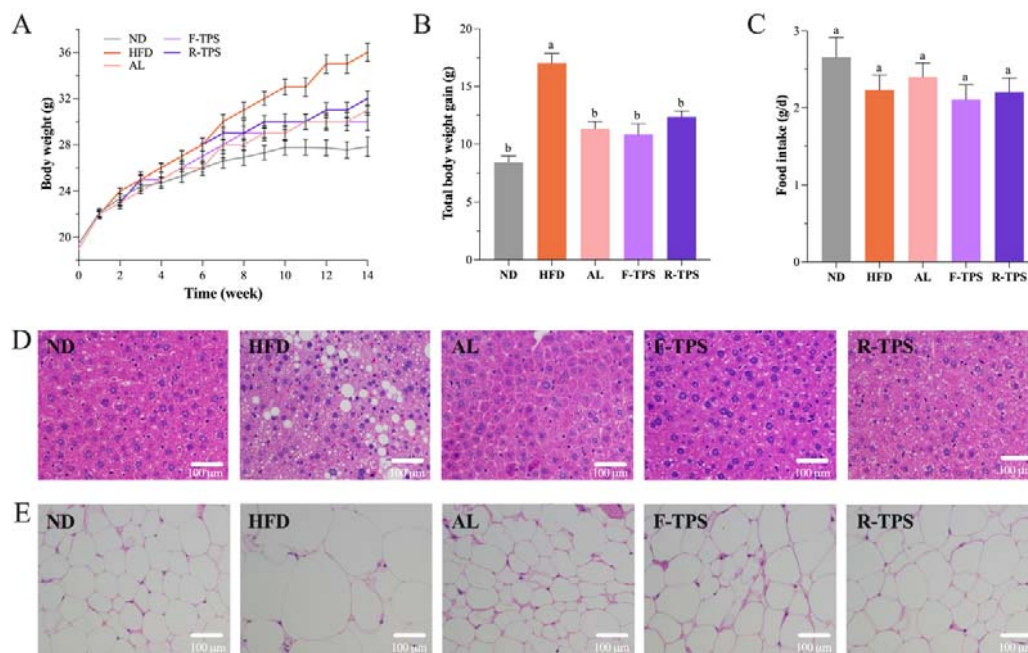
UV spectroscopy was employed to assess the presence of proteins and nucleic acids in F-TPS and R-TPS, while FT-IR spectroscopy was used to characterize their functional groups. As shown in Fig. S1b, both F-TPS and R-TPS displayed no obvious absorption peaks at 280 and 260 nm, indicating the absence of nucleic acids and proteins in these polysaccharide samples. The FT-IR spectra of F-TPS and R-TPS (Fig. S1c), showed a broad and intense absorption band at 3404 and 3937 cm^{-1} , which corresponds to O–H stretching vibrations. A peak observed at 2928 cm^{-1} was attributed to the asymmetric C–H stretching vibration of carbohydrates. The peak at 1614 cm^{-1} was assigned to the asymmetric stretching vibration of C=O groups, while the peak at 1411 cm^{-1} corresponded to C–O stretching. The region between 1411 and 1040 cm^{-1} exhibited variations in peak patterns, likely due to differences in monosaccharide composition and content between the two polysaccharides. In addition, strong absorption bands around 1040 cm^{-1} were associated with the C–O–C

stretching of ether linkages in the pyranose ring and O–H bending vibrations. These FT-IR results confirm that both F-TPS and R-TPS are heteropolysaccharides.

3.2 F-TPS and R-TPS ameliorated the obesity parameters of obese mice

In general, increased body weight, tissue indexes, serum lipid, and blood glucose levels are direct indicators of obesity, therefore, these obesity-related parameters were measured in the present study. As shown in Fig. 1A, the body weight of HFD group was the highest, researching to 36.45 g, while ND group had a body weight of 27.84 g. The growth trends of the R-TPS and F-TPS groups closely mirrored that of the ND group, with final body weights reaching 31.78 g and 30.26 g, respectively, by the end of the experiment. Similar trends were also observed in body weight gain across the groups. The HFD group exhibited the highest weight gain at 17.02 g, while the R-TPS and F-TPS groups showed significantly reduced weight gains of 12.35 g and 10.83 g, respectively. These findings indicate that R-TPS and F-TPS effectively mitigated excessive body weight gain in obese mice (Fig. 1A&B). Generally, weight gain leads to the accumulation of fat, resulting in the increased tissue indexes. The results in Table S2 demonstrated that the intervention of R-TPS and F-TPS remarkably decreased the tissues indexes, thus, ameliorated body fat storing. Notably, the food intake did not show significant difference between all the HFD-fed mice, suggesting the alleviation of body weight and fat accumulation was not contribute to reduced appetite (Fig. 1C). Moreover, hepatic and lipid histology analysis in Fig. 1D-F indicated that HFD supplement led to increased lipid droplets accumulation and the appearance of hepatocellular ballooning degeneration and inflammatory cell infiltration. HFD consumption also significantly promoted adipose cell hypertrophy and enhanced adipocyte area. However, R-TPS and F-TPS intervention could efficiently alleviate the process of fat accumulation, leading to reduction of the hepatocellular balloon-like structure, lipid deposition, adipocyte size, and adipocyte area. In addition, similar trends were found in OGTT, ITT, and HOMA-IR results. As shown in Fig. 1G-L, HFD treatment remarkably broken the serum glucose hemostasis and induced insulin resistant in obese mice. After being fed with R-TPS and F-TPS, the impaired glucose and insulin regulation were greatly improved with remarkably reduced AUC of ITT and OGTT and HOMA-IR in the obese mice. And F-TPS intervention was more effective than R-TPS in balancing blood glucose homeostasis. As shown in Fig. 1M, in comparison of ND group, HFD group mice possessed much higher serum lipid levels of TC, TG, and LDL-c and lower content of HDL-c ($P < 0.05$), suggesting the long term HFD feeding could cause lipid metabolism disorder. R-TPS and F-TPS administration dramatically reduced the levels of TC, TG, and LDL-c and increase the level of HDL-c, and the effect of R-TPS and F-TPS were almost the same. Additionally, HFD administration significantly increased the content of FFA, reaching (3.42 ± 0.38) mmol/L, while that of ND group was (2.05 ± 0.1) 4 mmol/L. However, the levels of FFA were decreased by 26.02% and 35.96% in the R-TPS and F-TPS groups, respectively, with compare with HFD group. Notably, R-TPS and F-TPS intervention also observably downregulated the hepatic concentration of TC and TG in comparison with HFD feeding, indicating R-TPS and F-TPS supplement could alleviate the HFD-induced abnormal lipids metabolism. Moreover, the liver function parameters including ALT, AST, AKP, and ACP were also detected. As shown in Table S3, HFD

feeding increased the serum ALT and AST contents by 1.57- and 1.42-fold, respectively, in comparison with normal diet feeding. However, R-TPS and F-TPS treatment memorably reduced the levels of ALT and AST by 29.45% and 31.86% and 19.24% and 22.01%, respectively, compared with those of the HFD group. The serum contents of AKP and ACP also showed similar trends. These results indicated R-TPS and F-TPS intervention could protect hepatic function in obese mice. In addition, the levels of serum endoxemia and pro-inflammatory cytokines were analyzed to evaluate the effect of R-TPS and F-TPS on the systemic chronic inflammation in HFD-fed mice. As depicted in Fig. 2, the levels of LPS, TNF- α , IL-6, and IL-1 β in the HFD group increased by 1.91-, 1.23-, 1.47-, and 1.33-fold, respectively, compared to the ND group, suggesting that long-term HFD feeding could lead to systemic inflammation. Following intervention with R-TPS and F-TPS, the serum contents of LPS, TNF- α , IL-6, and IL-1 β were observably reduced by 54.28%, 9.91%, 8.33%, and 23.71%, respectively, in the R-TPS group, and by 46.26%, 24.11%, 11.71%, and 29.66%, respectively, in the F-TPS group ($P < 0.05$). These findings suggest that both R-TPS and F-TPS could attenuate the chronic low-grade inflammation in the HFD-induced obese mice, and F-TPS showed more effect in reducing TNF- α , IL-6, and IL-1 β , while R-TPS was more efficient in decreasing LPS.



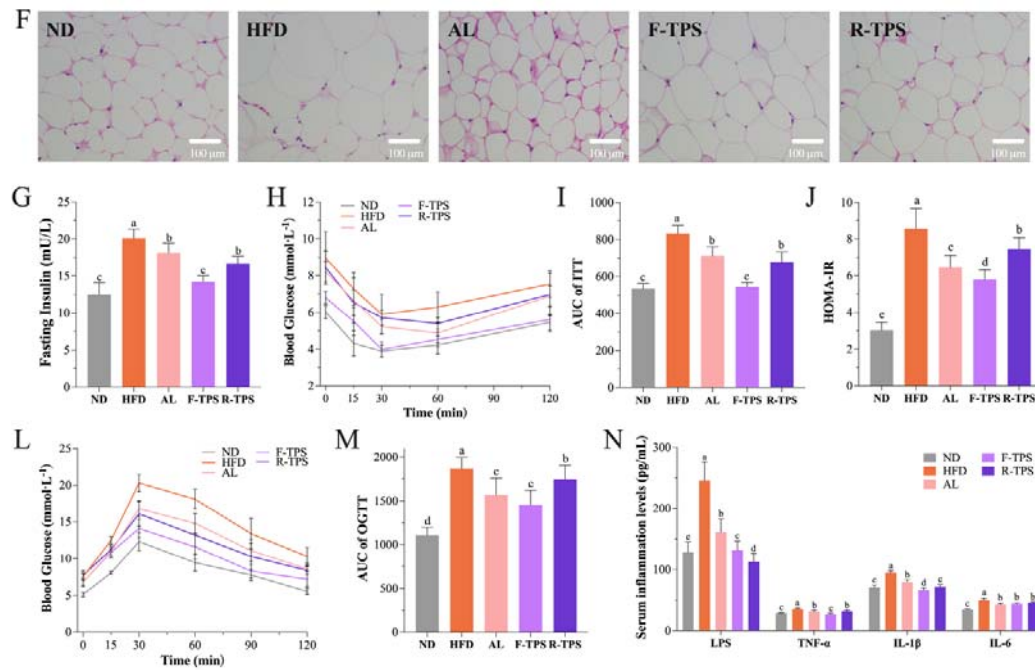
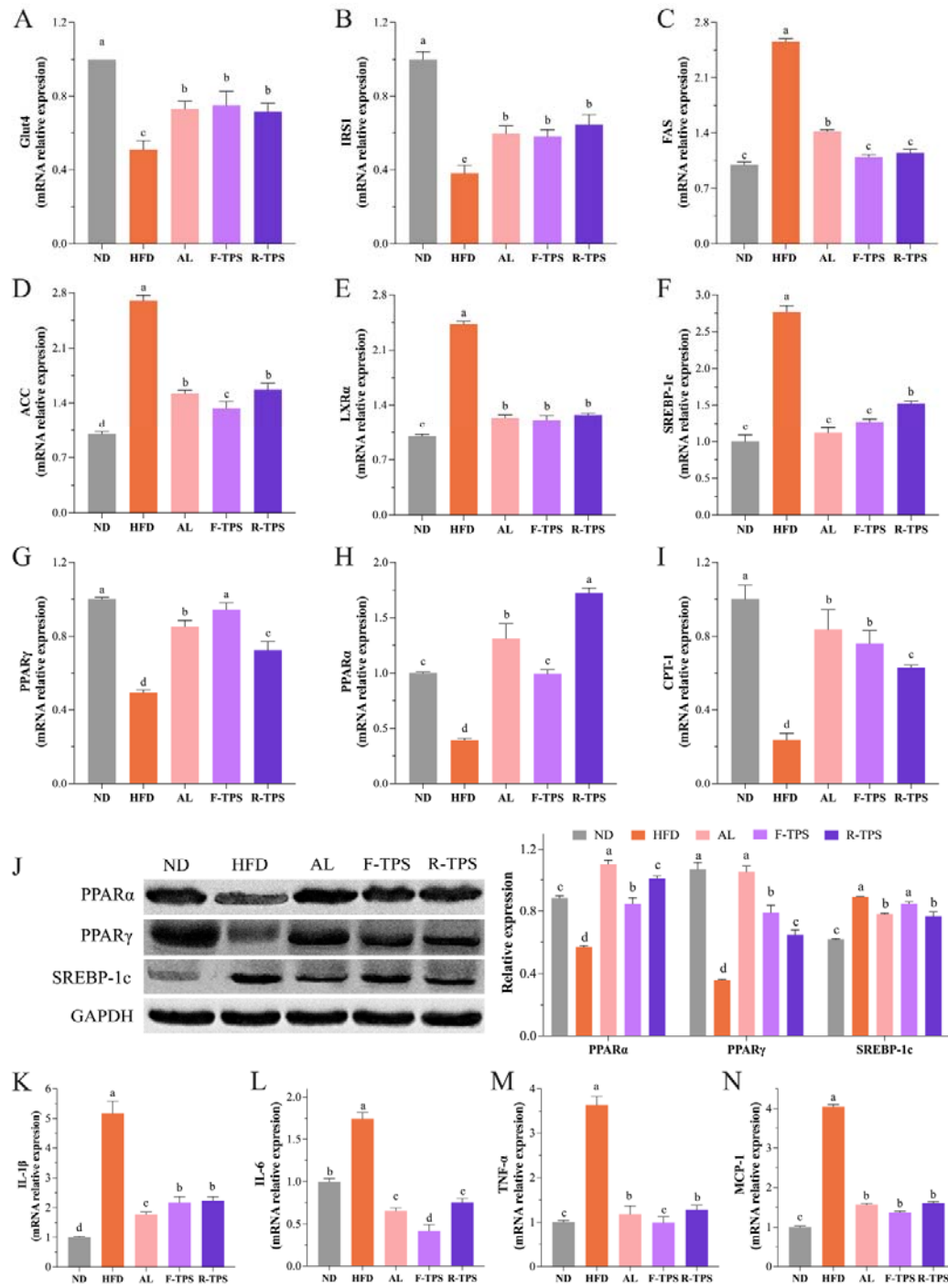


Fig. 1. The effects of R-TPS and F-TPS on HFD-induced obese mice.

A. body weight; B. body weight gain; C. food intake; D-F. representative photographs of the liver, epididymal fat and perirenal fat; G. fasting insulin; H. ITT; I. AUC of ITT; J. HOMA-IR; K. OGTT; L. AUC of OGTT; M. the levels of serum endoxemia and pro-inflammatory cytokines. The bar graphs with different letters on top represent statistically significant results ($P < 0.05$).

3.3 R-TPS and F-TPS adjusted the expression of genes and protein related to glucose and lipid metabolism

The effect of R-TPS and F-TPS on the expression levels of genes related to glucose and lipid metabolism was analyzed. The results depicted in Fig. 2A-B indicated that HFD consumption profoundly downregulated the mRNA expression of Glut4 and IRS. Intervention with R-TPS and F-TPS remarkably reversed the HFD-induced suppression of Glut4 and IRS1 expression. In addition, the mRNA levels of FAS, ACC, LXR- α , and SREBP-1c in the HFD group was markedly upregulated by 2.56-, 2.70-, 2.43-, and 2.77-fold, respectively, compared to the ND group. Conversely, the levels of CPT-1, PPAR γ , and PPAR α were notably downregulated to 23.66%, 49.21%, and 39.23% of the ND group, respectively. However, administration of R-TPS and F-TPS substantially reversed these HFD-induced variations, restoring gene expression levels close to the levels of those of the ND group. Noticeably, the F-TPS group exhibited significantly higher expression of CPT-1 and PPAR γ and lower the levels of ACC, SREBP-1c, and PPAR α compared to the R-TPS group ($P < 0.05$). For the proteins related to lipid metabolism, the HFD group showed remarkably lower levels of PPAR α and PPAR γ , alongside higher expression of SREBP-1c, compared to the ND group. R-TPS and F-TPS intervention observably reversed the expression levels of those lipid metabolism related proteins, aligning these proteins expression close to those of the ND group, and F-TPS is more efficient than R-TPS in upregulating PPAR α and PPAR γ protein expression. The abovementioned data suggested that R-TPS and F-TPS play essential roles in maintaining normal glucose and lipid metabolism.



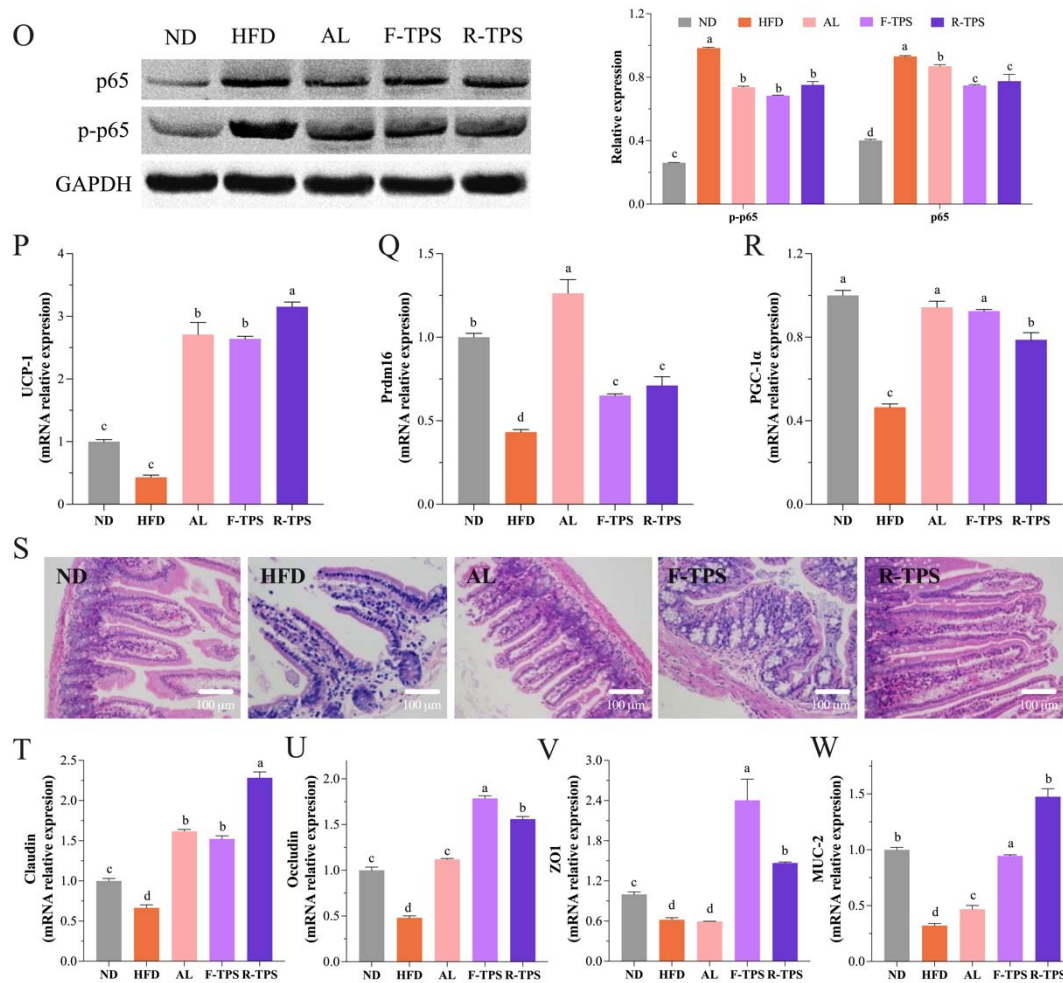


Fig. 2. The effects of R-TPS and F-TPS on the expression of genes and protein related to glucose and lipid metabolism.

A-B. the mRNA expression of Glut4 and IRS1; C-I. the mRNA expression of FAS, ACC, LXRα, SREBP-1c, PPARγ, PPARα, and CPT-1; J. the protein expression of PPARα, PPARγ, and SREBP-1c; K-N. the mRNA expression of IL-1β, IL-6, TNF-α, and MCP-1; O. the protein expression of p65, p-p65; P-R. the mRNA expression of UCP-1, PRDM16, and PGC-1α.; S. representative histological micrographs of colon; T-W. the mRNA expression of MUC-2, ZO-1, occluding, and claudin. The bar graphs with different letters on top represent statistically significant results ($P < 0.05$).

To further explore the effects of R-TPS and F-TPS on inflammation in HFD-induced obese mice, the expression levels of IL-1β, IL-6, TNF-α, and MCP-1 in liver tissues were analyzed, with the results shown in Fig. 2K-N. Compared to the ND group, the mRNA expression of the abovementioned pro-inflammatory factors in the HFD group was significantly upregulated by 5.17-, 1.74-, 3.64-, and 4.05-fold, respectively ($P < 0.05$). In contrast, the expression levels of these inflammatory factors in the liver of mice in R-TPS and F-TPS group was inhibited to a certain extent, suggesting that R-TPS and F-TPS may mitigate tissue infiltration and the secretion of pro-inflammatory cytokines in the obese mice, and the effect of F-TPS was better than R-TPS. The expression of p65 and phosphorylated p65 (P-p65) protein in the NF-κB pathway, which is an important inflammatory signaling pathway, was further assessed, and the results are shown in Fig. 2O. As shown, the protein levels of p65 and P-p65 in liver of the HFD-induced obese mice were markedly elevated ($P < 0.05$), while those in the groups receiving R-TPS and F-TPS were considerably downregulated, indicating R-TPS and F-TPS administration exerted anti-inflammatory effect by the regulation of the NF-κB pathway.

Although F-TPS performed better than R-TPS, there was no significant difference between the two in regulation the protein expression.

The expression level of genes related to lipid browning in white adipose tissue of obese mice can serve as an important indicator for investigating the anti-obesity effects of R-TPS and F-TPS. The results in Fig. 2P-R showed that HFD feeding significantly downregulated the expression of UCP-1, PRDM16, and PGC-1 α mRNAs, impairing the ability to dissipate excess energy as heat, thereby promoting fat storage. However, the administration of R-TPS and F-TPS significantly upregulated the expression of these browning-related genes ($P < 0.05$), suggesting that both treatments enhance adipose tissue browning and energy expenditure. And F-TPS was more efficient than R-TPS in promoting UCP-1 and PGC-1 α gene expression

3.4 R-TPS and F-TPS restored the impaired colon tissue in obese mice

The intestinal morphology can visually reflect the integrity of the intestinal mucosa. To explore the protective effects of R-TPS and F-TPS administration on the intestinal morphology of obese mice induced by long-term HFD feeding, H&E staining of colonic tissues was performed. As shown in Fig. 2S, compared to the ND group, the intestinal integrity of the HFD group was severely damaged due to the prolonged HFD treatment, with sparse and shorter intestinal villi. In contrast, the R-TPS and F-TPS groups showed dense and higher intestinal villi, and a thicker submucosal layer.

The relative expression levels of mucins MUC-2, ZO-1, occluding, and claudin genes, which are critical for intestinal barrier function, were also examined. As shown in Fig. 2T-W, compared to the ND group, the expression of intestinal MUC-2, ZO-1, occludin, and claudin was significantly lowered by 32.15%, 61.99%, 48.00%, and 66.56%, respectively, in the HFD group ($P < 0.05$), indicating that HFD feeding impaired the integrity of the intestinal mucosa. However, R-TPS and F-TPS administration effectively restored the expression of these genes and enhanced the intestinal barrier integrity in mice on HFD. F-TPS is more effective than R-TPS in promoting the gene expression of Occludin and ZO-1, whereas R-TPS shows greater efficiency in enhancing the expression of Claudin and MUC-2. These results suggest that R-TPS and F-TPS can effectively ameliorate the damage to colonic tissues and protect the integrity of intestinal barrier, promoting intestinal homeostasis in obese mice.

3.5 R-TPS and F-TPS ameliorate the production of SCFAs in obese mice

SCFAs, the primary metabolites of gut microbiota, play a vital role in maintaining human health. To assess the effects of R-TPS and F-TPS on the production of SCFAs, the cecal SCFAs contents were measured. The results in Table S4 indicated that ND group exhibited the highest total cecal SCFAs content at $(162.22 \pm 2.2) \mu\text{mol/g}$, whereas HFD group possessed the lowest SCFAs content at $(39.04 \pm 0.65) \mu\text{mol/g}$. Acetic acid, propionic acid, and isobutyric acid were the main components of SCFAs, with acetic acid dominating the profile of SCFAs, accounting for more than 80% of total SCFAs. Compared to the HFD group, R-TPS and F-TPS administration could significantly promote the generation of SCFAs. Surprisingly, mice with R-TPS supplementation resulted in remarkable higher levels of total SCFAs than F-TPS treatment, specifically, R-

TPS administration led to much higher contents of acetic acid, butyric acid, isobutyric acid, and hexanoic acid, while mice with F-TPS treatment had higher content of propionic acid ($P < 0.05$).

3.6 R-TPS and F-TPS remodeling gut microbiota

Gut microbiota has been shown to play a vital role in the pathogenesis of obesity and its related complications. To elucidate the mechanism of R-TPS and F-TPS in ameliorating obesity, the bacterial 16S rRNA was conducted in the present study to investigate the effects of R-TPS and F-TPS on gut flora composition. After filtering out unqualified sequences, the average number of reads exceeded 482,192 per sample, resulting in 242 OTUs with the homology truncation value of 97%. The rarefaction curve demonstrated that the sequencing depth covered a wide range of rare novel phylotypes, capturing the majority of the microbial diversity present (Fig. S2).

ACE, Chao1, Shannon and Simpson indices were used for α -diversity analysis to evaluate the diversity of microbial species in samples. Fig. 3A-D showed that HFD feeding dramatically brought down Chao1, ACE, and Shannon indices, while critically enhanced Simpson index, suggesting that HFD feeding might lead to the dysbiosis of gut microbiota characterized by decreased bacterial abundance and diversity. However, R-TPS and F-TPS administration effectively reversed the reduction in bacterial richness and diversity induced by the HFD feeding, restoring the α -diversity indices to levels similar to those of ND group. Moreover, the Venn diagram analysis results shown in Fig. 3E demonstrated that 229 OTUs were common in all the five groups. The ND group possessed the 30 unique OTUs, followed by the F-TPS, R-TPS, AL, and HFD groups with 52, 65, 126, and 106 OTUs, respectively.

Meanwhile, principal coordinate analysis (PCoA) showed that the structure of intestinal flora changed significantly after long-term feeding of HFD, and there was a significant difference between the HFD and ND groups. While the F-TPS, R-TPS, and AL intervention groups were distant from the HFD group and tended to cluster with the ND group, respectively, suggesting that F-TPS and R-TPS could potentially remodel the HFD-damaged intestinal flora structure toward ND group (Fig. 3F).

In the present study, we further analyzed the intestinal flora structure of the five groups to gain a comprehensive understanding of the effects of HFD feeding and R-TPS and F-TPS intervention on the composition and abundance of intestinal flora. As could be seen in Fig. 3G & H, the samples of each group at the phylum level mainly included Firmicutes, Bacteroidetes, Actinobacteria, and Verrucomicrobia, among which Firmicutes (34.50%-75.24%) and Bacteroidetes (7.10%-18.81%) were the dominant in each group, while Verrucomicrobia was more prevalent in the ND group (7.08%), F-TPS (5.68%), and R-TPS (25.29%) groups and less in HFD (1.58%) and AL (0.23%) groups. Actinobacteria were less in ND, AL, and R-TPS groups with the proportion of 3.21%, 2.54% and 3.85% respectively, while in HFD group it was substantially increased to 34.89%. Proteobacteria were 4.72% in HFD group and little in other groups. The rest including Patescibacteria, Epsilonbacteraeota, Cyanobacteria, Tenericutes, Deferribacteres were less in all groups, which corroborated with previous studies.^[2]

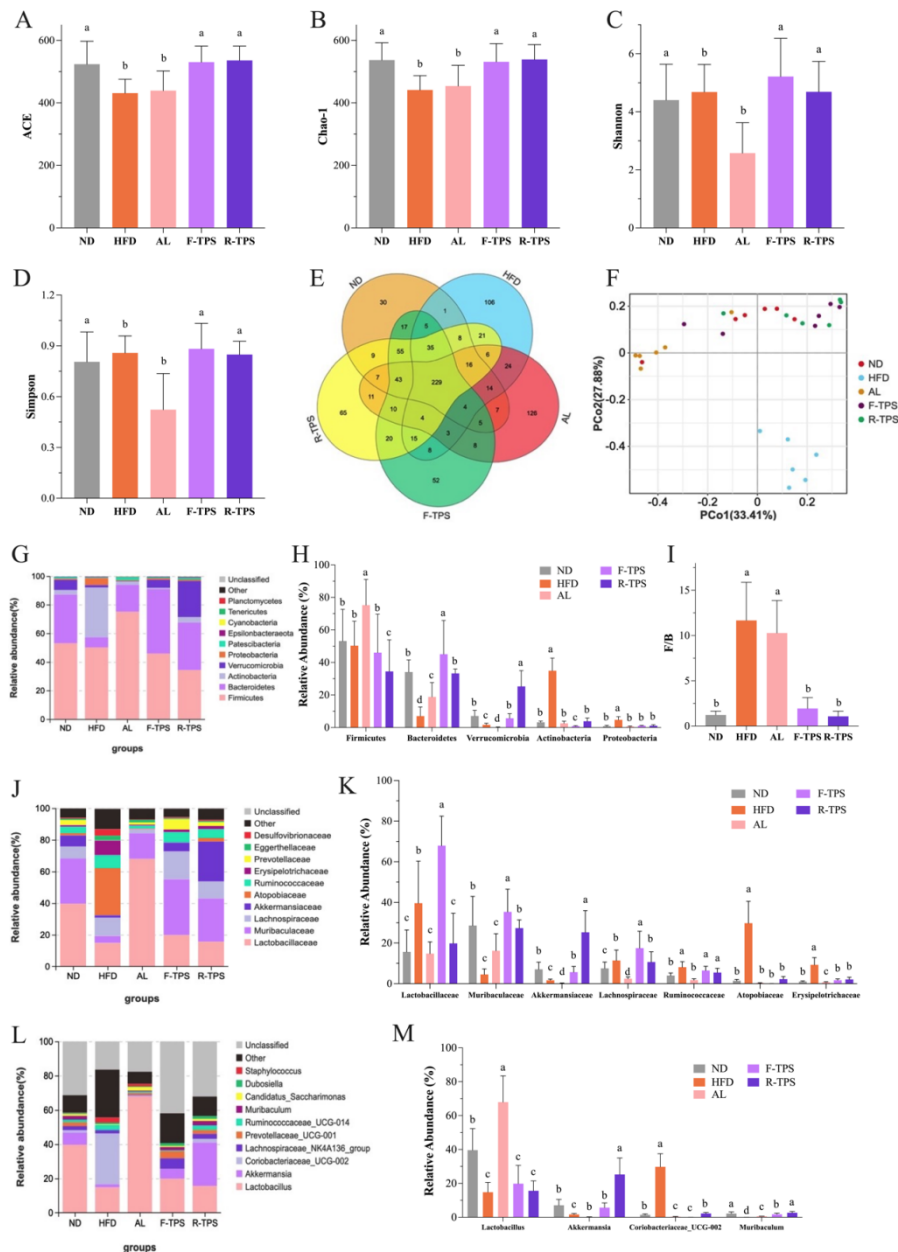


Fig. 3 The effect of R-TPS and F-TPS on the HF D-induced gut inflora dysbiosis.

A. ACE index; B. Chao-1 index; C. Shannon index; D. Simpson index; E. Venn plot; F. PCA score plot; G. bacterial taxonomic profiling of intestinal flora at the phylum level; H. the relative abundances of five representative phylum; I. the ratio of Firmicutes to Bacteroidetes; J. gut microbiota at the family level; K. relative abundance of six representative families; L. gut microbiota at the genus level; M. relative abundance of four representative genera. The bar graphs with different letters on top represent statistically significant results ($P < 0.05$).

The ratio between species at the phylum level is also an important examination index of the gut microbial structure, in which the ratio of Firmicutes and Bacteroidetes (Firmicutes/Bacteroidetes, F/B) is considered to be an essential measure of gut microbial dysbiosis, which can be seen in Fig. 3I, whereas the ratio of F/B in the HFD group was 7.15, significantly higher than that of 1.56 in the ND group ($P < 0.01$), and the F-TPS and R-TPS administration significantly reduced the F/B value to 1.02 and 1.04 ($P < 0.05$), respectively. As we can see from Fig. 3H, the percentage of Verrucomicrobia in the HFD group of mice decreased substantially relative to ND group, whereas F-TPS and R-TPS treatment was effective in increasing the proportion of Verrucomicrobia in the gut microbiota of obese mice, especially, R-TPS supplement boosted it to 15-fold.

The relative abundance of Actinobacteria and Proteobacteria in the HFD group was significantly higher compared with that of the ND group, which suggested that a long-term HFD significantly increased the relative abundance of Actinobacteria and Proteobacteria in gut microbes. F-TPS and R-TPS administration could effectively inhibit the increase of Actinobacteria and Proteobacteria and maintained the homeostasis of intestinal microbial structure.

The distribution of major species at family level in each group is shown in Fig. 3K. The main species at family level include Lactobacillaceae, Muribaculaceae, Lachnospiraceae, Akkermansiaceae, Ruminococcaceae, Prevotellaceae, etc. The proportions of Lactobacillaceae and Muribaculaceae in the HFD group were 39.61% and 4.50%, dramatically different from 15.56% and 28.61% in the ND group. Notably, F-TPS treatment critically enhanced the relative abundance of Lactobacillaceae and Muribaculaceae to 67.93% and 35.32%, while those in the R-TPS were 19.82% and 27.36%, respectively, very close to the ND group. In addition, there were obvious differences in the distribution of species at family level in the HFD group compared with the other groups, with a large number of Atopobiaceae in the HFD group, accounting for 29.80% of the total, and only 0.062%-2.23% of the species in the other groups. Similarly, the relative abundance of Erysipelotrichaceae was 9.28% in the HFD group, while varied from 0.71% to 2.11% in the other groups. In contrast, Akkermansiaceae was abundant in the R-TPS group with a percentage of 25.28%, and also had a large proportion in the ND (7.08%), and F-TPS (5.69%), but its presence in the HFD group was only 1.68%. Interestingly, Lachnospiraceae was also found to account for 11.41% in HFD group, much higher than 7.52% in the ND group, F-TPS intervention remarkably promoted the proliferation of Lachnospiraceae, however, R-TPS had little effect on it. These results indicate that the species distribution of intestinal microorganisms at family level in the HFD group of mice was significantly different from that of the R-TPS and F-TPS administration groups.

The species distribution of the samples in each group at genus level is shown in Fig. 3M, where *Lactobacillus* (15.64% - 67.93%) and *Akkermansia* (1.68% - 25.28%) were the dominant species in each group. Compared to the ND group, *Lactobacillus* content in the HFD group decreased to 35.46%, while it increased 1.45-fold in the F-TPS group and 1.12-fold in the R-TPS group. *Coriobacteriaceae_UCG-002* was abundantly present in the HFD group only, with a proportion of 29.80%, while it was less in the ND, AL, F-TPS, and R-TPS groups, with a proportion of 1.44%, 0.33%, 0.06%, and 2.22%, respectively. The proportion of *Lachnospiraceae_NK4A136_group* was 2.00 % in the HFD group, R-TPS treatment had little influence on the abundance of it, notably, F-TPS administration significantly increased the abundance of *Lachnospiraceae_NK4A136_group* to 6.20%. The abundance of *Prevotellaceae_UCG-001* was only account for 0.03% in the HFD group, while it was increased to 2.32% and 4.24% after R-TPS and F-TPS treatment, the former was very close to that of ND group. And the percentage of *Akkermansia* in HFD group was only 1.68%, which was much less than 25.28% in R-TPS group, 7.08% in ND group and 5.69% in F-TPS group. The administration of R-TPS and F-TPS could significantly promote the increase in the abundance of *Akkermansia* in mice intestine fed HFD, suggesting that R-TPS and F-TPS might facilitate the increase of

Akkermansia, thereby reducing the body's utilization of energy and inhibiting obesity, and also promote the renewal of intestinal surface cells to protect the integrity of the intestinal barrier. *Muribaculum* was only present in a very small amount in the HFD group, accounting for only 0.03%, but was more prevalent in the ND, F-TPS, R-TPS, and AL groups, accounting for 2.20%, 1.79%, 2.65% and 0.59%, respectively. F-TPS and R-TPS administration increased the abundance of *Muribaculum* compared to HFD group, suggesting that the mechanism of F-TPS and R-TPS for weight loss may be related to the promotion of *Muribaculum*.

To clarify the bacterial taxa significantly regulated by HFD and F-TPS and R-TPS treatments, LEfSe (LDA > 4) analysis was used to compare the microbiota composition of these groups. As could be seen in Fig. 4A, totally 48 distinct taxa were figured out among the five groups. Specifically, ND group possessed *Lactobacillus_gasseri* as the only dominant difference species. In comparison, HFD group had 19 significantly diverged species, with Atopobiaceae, Erysipelotrichaceae, and Coriobacteriaceae as predominant families. F-TPS group was characterized by 12 advantageous taxa served as biomarkers, with *Muribaculaceae*, *Lachnospiraceae*, *Lachnospiraceae_NK4A136_group*, and *Prevotellaceae_UCG_001* as dominant genera. Whereas, the R-TPS group also positively identified with 10 traits such as Akkermansiaceae, Halieaceae, *Muribaculum*, and *Persicirhabdus*. Thus, these bacteria can be used as biomarkers in F-TPS- and R-TPS-fed mice and served as effective indicators of improvement in HFD-induced obesity.

3.7 Correlation between F-TPS and R-TPS Remodeling Gut Microbiota and Biochemical Parameters

Spearman's correlation analysis on level of species was conducted to identify the potential correlation between bacteria species with significant changes in gut flora and measured obesity-related traits. As can be seen from Fig. 4B, changes in body and organ weight, serum lipids, lipid metabolism-related genes, inflammatory cytokines, and SCFAs were significantly influenced by changes in the composition of the gut flora. The top twenty species in abundance at genus level were screened for their association with obesity parameters. The heatmap showed that *Coriobacteriaceae_UCG-002*, *Ruminococcaceae_UCG-014*, *Staphylococcus*, *Bifidobacterium*, *Enterorhabdus*, and *Allobaculum* correlated significantly and positively with body weight, liver weight, epididymal fat, perirenal fat, TC, LDL-c, TNF- α , IL-1 β , and UCP1, and statistically negatively with PPAR α , PPAR γ , claudin, occludin, MUC-2, and ZO-1. In contrast, *Akkermansia*, *Prevotellaceae_UCG-001*, and *Muribaculum* were negatively correlated with body weight, liver weight, epididymal fat, perirenal fat, TC, LDL-c, insulin, blood glucose, SREBP-1c, FAS, LXR, ACC, TNF- α , IL-1 β , MCP1, and UCP1, while positively correlated with HDL-c, Glut4, IRS1, CPT1, PPAR α , PPAR γ , claudin, occludin, muc2, and ZO1. The results of spearman's analysis suggested that different gut microbes varied dramatically in the effect on host. However, it is important to note that these correlations reflect statistical associations rather than direct biological causality.

significantly different from those in the ND-F group. Compared with the ND-F group, HFD-F treatment resulted in an obvious enhancement of the serum level of LPS, whereas F-TPS and R-TPS FMT treatments led to evident lower serum LPS levels (Fig. 5I).

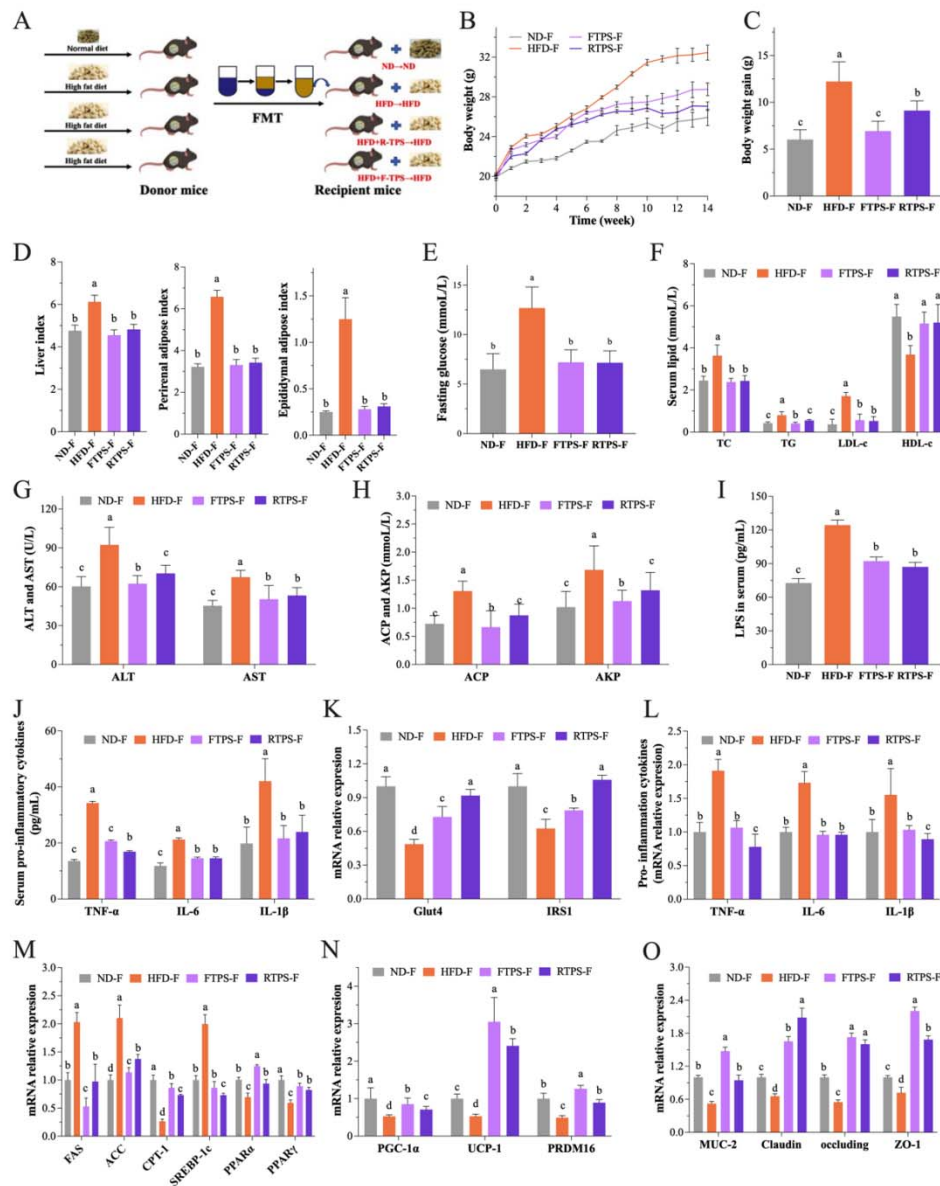


Fig. 5 Amelioration of HFD-induced obesity in donor mice by R-TPS and F-TPS.

A. Experimental scheme; B. body weight; C. body weight gain; D. tissue indexes; E. fasting glucose; F. serum lipid including TC, TG, LDL-C, and HDL-C; G. serum ALT and AST; H. serum ACP and AKP; I. serum LPS; J. serum pro-inflammation cytokines; K. relative mRNA expression of Glut4 and IRS1; L. relative mRNA expression of pro-inflammation cytokines; M. relative mRNA expression of lipid metabolism related genes; N. relative mRNA expression of lipid browning related genes; O. relative mRNA expression of intestinal integrity related genes. The bar graphs with different letters on top represent statistically significant results ($P < 0.05$).

The effect of F-TPS and R-TPS FMT treatment on the systemic inflammation, glucose metabolism, lipid metabolism, adipose browning, and the intestinal integrity in recipient mice were further examined (Fig. 5J-O). In comparison with HFD-F group, the FTPS-F, RTPS-F, and ND-F groups possessed dramatic lower serum IL-6, IL-1 β , and TNF- α . Similarly, the mRNA expression levels of IL-6, IL-1 β , and TNF- α showed the similar trends in all the four groups. HFD FMT processing dramatically down-regulated the expression of Glut4 and IRS1, whereas the expression levels of these two genes were considerably up-regulated, and

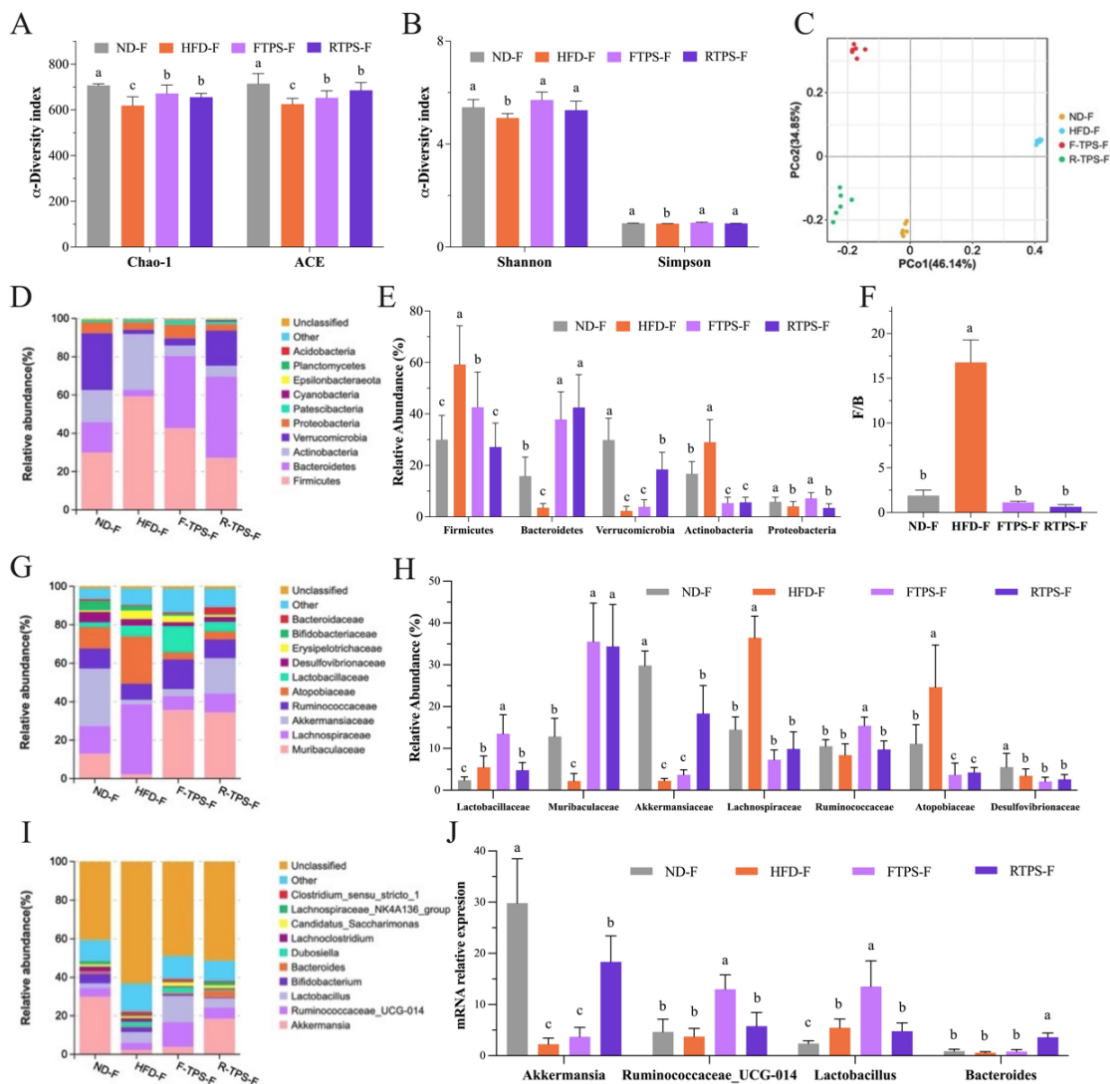
notably, R-TPS FMT processing restored the impaired glucose metabolism to normal level. In addition, the expression of FAS, ACC, and SREBP-1c was apparently elevated in mice in the HFD-F group, and the expression levels of CPT-1, PPAR γ , and PPAR α were lower, and the levels of these lipid metabolism-related genes were reversed to those of the ND-F group after F-TPS and R-TPS FMT treatments. Intriguingly, R-TPS FMT therapy led to enhanced levels of FAS and ACC, whereas F-TPS FMT treatment had higher CPT-1, SREBP-1c and PPAR α expression. Similarly, F-TPS and R-TPS FMT processing upregulated gene expressions of PGC-1 α , UCP1, and PRDM16, suggesting WAT browning was promoted and BAT thermogenesis was activated in FTPS-F and RTPS-F groups. And FTPS-F group had markedly higher expression levels of these three genes than RTPS-F group. Additionally, the levels of genes related to intestinal barrier integrity including MUC-2, claudin-1, occluding, and ZO-1 were investigated in the FMT assay. The FTPS-F, RTPS-F, and ND-F recipient mice gained higher levels of the abovementioned three genes than the HFD-F mice, indicating that FTPS and RTPS FMT could ameliorate the damaged gut mucosal barrier of the recipient mice. Together, F-TPS and R-TPS FMT effectively ameliorated HFD-induced obesity and the related features, low-grade systemic inflammation, lipid metabolism, adipose browning, and intestinal barrier integrity.

3.9 FMT from F-TPS- and R-TPS-administered donor mice regulated intestinal dysbiosis in HFD-Fed recipient mice

The present study further examined the gut microbiota of the HFD-Fed recipient mice. The HFD FMT treatment resulted in a remarkable decrease in the α -diversity indexes including Chao-1, ACE, Shannon, and Simpson in the recipient mice (Fig. 6A&B). The FTPS and RTPS FMT processing markedly enhanced these indexes, turning them close to those of ND-F group. Additionally, there were statistical differences in the structure of gut microbiota between FTPS-F and RTPS-F groups and the HFD-F group according to the PCoA scoring plot (Fig. 6C).

At phylum level, Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia, and Proteobacteria constituted the five dominant phyla of each group (Fig. 6D&E). Compared with ND FMT treatment, HFD FMT treatment obviously increased the abundance of Firmicutes and the ratio of F/B and decreased the proportion of Bacteroidetes (Fig. 6F). FTPS and RTPS FMT treatment dramatically restored the abovementioned changes in the recipient mice, which was similar to the effect of F-TPS and R-TPS supplementation in the donor mice. At family level, Muribaculaceae, Lachnospiraceae, Akkermansiaceae, Ruminococcaceae, Atopobiaceae, Lactobacillaceae, Desulfovibrionaceae, and Erysipelotrichaceae were the predominant families in all the four groups (Fig. 6G&H). A dramatic reduction of the relative abundance of Muribaculaceae, Akkermansiaceae, and Ruminococcaceae and an observable enhancement of Lachnospiraceae and Atopobiaceae were observed in the HFD-F group in comparison with ND-F group. Unsurprisingly, these changes were memorably reversed by FTPS and RTPS FMT treatment, and the distribution and the abundance of the families in all the recipient groups were also similar to the donor groups. At genus level, the predominant genera were *Akkermansia*, *Ruminococcaceae_UCG-014*, and *Lactobacillus*

(Fig. 6I&J). The proportion of *Akkermansia* was signally down-regulated from 29.81% in ND-F group to 2.25% after the HFD FMT treatment, compared to 3.71% and 18.35% under FTPS and RTPS FMT treatment, respectively. Compared with ND-F group, *Ruminococcaceae_UCG-014* was slightly reduced in the HFD-F group, while there were 3.46- and 1.54-fold increase in FTPS and RTPS groups, respectively. Intriguingly, the abundance of *Lactobacillus* was 5.47%, 2.29-fold increased after the HFD FMT treatment compared with ND FMT treatment, it was slight reduced to 4.80% in the RTPS-F group, while markedly increased to 13.51% in the FTPS-F group. No significant differences in the relative abundances of *Bifidobacterium* in the HFD-F, ND-F, and FTPS-F groups, while it was significantly increased to 3.59% in RTPS-F group, 6.09-fold higher than that in the HFD-F group. Although the composition of the intestinal flora of the four groups of recipient mice was not identical to that of the donor mice, the trends of the dominant genera in the FTPS-F and RTPS-F groups were similar to those of the F-TPS and R-TPS donor mice.



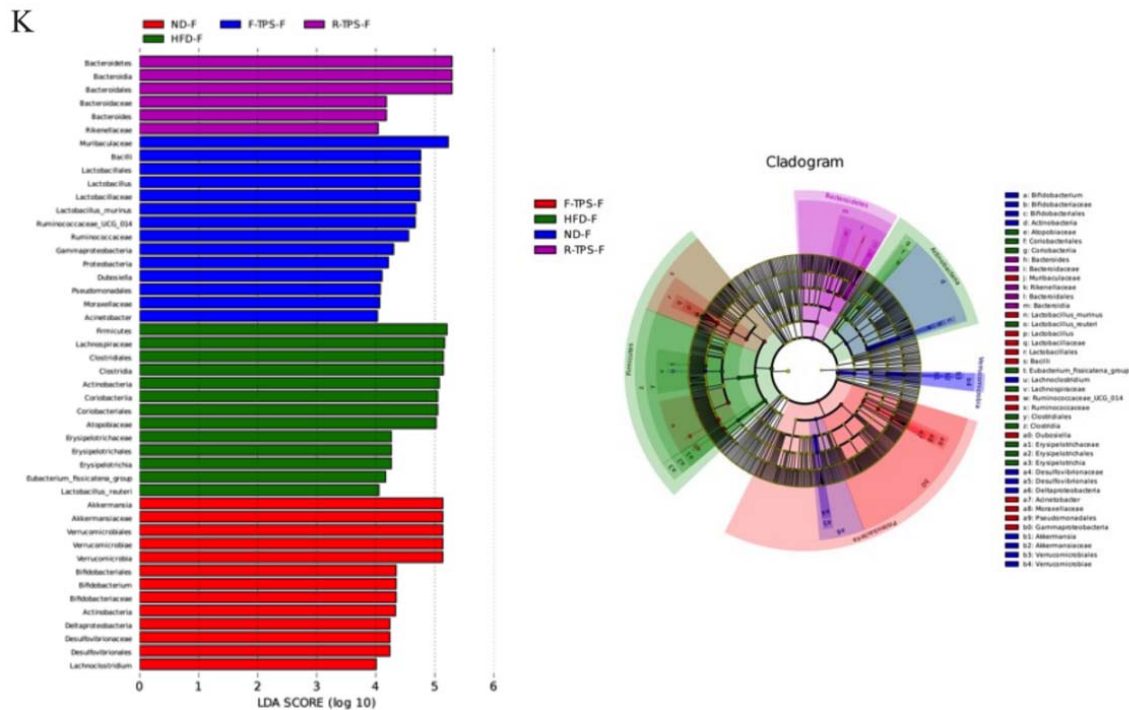


Fig. 6 Amelioration of HFD-induced intestinal flora dysbiosis by FMT.

LEfSe analysis was further conducted to reveal the predominant OTUs of each experimental group. Totally, 45 OUTs with LDA > 4 were identified in the four groups (Fig. 6K). The intestines of HFD-F group mice were enriched with 13 differentially significant species, including Lachnospiraceae, Atopobiaceae, Danubacteriaceae, *Clostridia*, *Eubacterium_fissicatena_group*, and *Lactobacillus_reuteri*. These families and genera also prevailed in HFD donor mice. The RTPS-F group was dominated by the genus *Bacteroides* of the family Bacteroides, whereas FTPS recipient mice treated with FTPS FMT had *Lactobacillus_murinus*, *Ruminococcaceae* UCG 014, *Acinetobacter* were all amplified.

To verify the colonization of donor microbiota, we analyzed the similarity between donor and recipient groups using PCoA and source tracking analysis. As shown in Fig. S3, the gut microbiota structure of recipient mice showed high similarity to their donors at the end of the 14-week transplantation period, particularly in the ND and HFD groups. For the intervention groups, although the R-TPS-F group displayed some separation from the R-TPS donor group, both F-TPS-F and R-TPS-F groups were distinctly separated from the HFD cluster and exhibited a consistent structural shift trend resembling their corresponding donors. This confirms that the specific gut microbiota signatures—specifically the reversal of HFD-induced dysbiosis—were successfully established and maintained until the endpoint in the recipient mice.

Therefore, both RTPS and FTPS FMT intestinal flora significantly ameliorated HFD-induced intestinal dysbiosis in receptor mice, and the intestinal flora of receiver mice resembled to some extent that of the corresponding donor mice.

4. Discussion

Accumulating studies have demonstrated that Pu-erh tea polysaccharides can lower body weight and regulate the intestinal flora, however, few studies have focused on how R-TPS and F-TPS differ in their

effects. Our study showed that supplementation with both R-TPS and F-TPS was effective in decreasing HFD-induced obesity and obesity-associated properties, such as body weight, weights of perirenal and epididymal fat, liver weight, tissue indexes, and histological appearances of adipose tissue and liver tissue. Earlier studies have indicated that tea polysaccharides such as green tea polysaccharides, black tea polysaccharides, white tea polysaccharides, and oolong tea polysaccharides also have anti-obesity effects.^[15-18] The anti-obesity potential might be a shared characteristic of polysaccharides extracted from different types of tea, despite their different chemical constituents. F-TPS was somewhat better than R-TPS in ameliorating body weight and organ indexes, but there was no significant difference between R-TPS and F-TPS. Supplementation of R-TPS and F-TPS also reduced fasting glucose levels, insulin contents, and HOMA-IR, and up-regulated the expression of genes related to glucose metabolism, with F-TPS regulating glucose homeostasis better than R-TPS. In addition, replenishment of R-TPS and F-TPS also reduced the increased lipid levels caused by HFD, improved liver function, inhibited the expression of genes and proteins related to lipogenesis, and promoted the expression of genes and proteins related to lipolysis, thus ameliorating obesity. R-TPS inhibited lipogenesis better than F-TPS, while F-TPS promoted lipolysis better. Additionally, R-TPS and F-TPS administration also reduced serum LPS and proinflammatory cytokines, suppressed the expression of inflammation-related genes as well as the expression and phosphorylation of proteins in HFD-fed mice, thus effectively attenuated the damage of the intestinal mucosal barrier and improved the integrity of the intestinal barrier. Although F-TPS was more effective than R-TPS in reducing serum LPS and inflammatory factor levels and downregulating the expression levels of related genes, there was no significant difference in the expression levels of important proteins in the NF- κ B inflammatory signaling pathway between R-TPS and F-TPS, suggesting that there may be other signaling pathways involved in the amelioration of inflammation in obese mice by R-TPS and F-TPS. Moreover, R-TPS and F-TPS could also remarkably up-regulate the expression of genes related to fat browning in mice and increase the body's thermogenesis to achieve weight loss. The results of the present study suggested that both R-TPS and F-TPS had anti-obesity activity, but they differed to different degrees in a variety of pathways, including ameliorating glucose and lipid metabolism, attenuating the inflammatory state in vivo, protecting the integrity of the intestinal barrier, etc.

In addition, R-TPS and F-TPS administration could significantly promote the generation of SCFAs. R-TPS administration led to much higher contents of acetic acid, butyric acid, isobutyric acid, and hexanoic acid, while mice with F-TPS treatment had higher content of propionic acid. Although SCFA receptors were not directly measured in this study, the elevated levels of specific SCFAs likely exert anti-obesity effects via G-protein coupled receptors (GPR41/43). For instance, the significant increase in butyrate observed in the R-TPS group is known to enhance intestinal barrier function and activate PPAR γ signaling. Meanwhile, the higher propionate levels in the F-TPS group may primarily regulate gluconeogenesis and insulin sensitivity via GPR43 activation in adipose tissues.

Considerable research has shown that the intestinal microbiota plays a key role in ameliorating obesity and related metabolic disorders through multiple mechanisms, such as inhibiting fat synthesis, promoting fat

metabolism, ameliorating chronic low-grade inflammation *in vivo*, and protecting the integrity of the intestinal barrier.^[5, 19-21] Regulating intestinal dysbiosis has been a key strategy in the prevention and treatment of obesity. The potential modulatory effects of R-TPS and F-TPS on the gut microbiota are not known, and whether there is a difference in their effects has not been reported. In the present study, feeding HFD led to a decrease in the α and β diversity of the gut microbiota compared with ND, which is consistent with previous studies.

Increasing evidences suggest that low microbial diversity is a hallmark characteristic of gut dysbiosis.^[2, 22] We found that HFD-induced decreases in α - and β -diversity were restored by R-TPS and F-TPS interventions. In addition, higher relative abundance of Firmicutes, lower abundance of Bacteroidetes, and significant increase in F/B ratio were also typical features of HFD-driven gut microbiota dysbiosis^[2, 23], and R-TPS and F-TPS interventions attenuated the abovementioned disorders, which is consistent with the effects of aqueous extracts of pu-erh tea we previously reported. Consistent with recent findings on other plant extracts^[24], our results confirmed that Pu-erh tea polysaccharides could ameliorate obesity by generally reversing HFD-induced dysbiosis (e.g., restoring the F/B ratio). However, unlike previous studies that often view anti-obesity as a singular outcome, our comparative analysis reveals a unique, fermentation-dependent specificity. We demonstrated that the distinct structural features of R-TPS and F-TPS dictate their specific microbial targets: R-TPS specifically enriches *Akkermansia* to reinforce the gut barrier and inhibit lipogenesis, whereas F-TPS promotes Muribaculaceae, Lachnospiraceae, and Lactobacillaceae to combat insulin resistance and systemic inflammation. This structure-microbiota-function specificity provides novel insights into how different forms of Pu-erh tea polysaccharides can be utilized for precise metabolic interventions.

Elevated endotoxin-producing Proteobacteria also reflected the disturbed intestinal microbiota.^[25] R-TPS and F-TPS supplementation reversed the HFD-induced increase in relative abundance of Proteobacteria and decrease in abundance of Verrucomicrobia, which may contribute to the improvement of LPS in the blood circulation. Verrucomicrobia has recently been recognized as a hallmark of a healthy gut because of its anti-inflammatory and immunostimulatory properties, as well as its ability to ameliorate intestinal barrier function and insulin sensitivity and reduce the level of endotoxemia.^[26, 27] The relative abundance of Verrucomicrobia in the intestines of mice in the R-TPS group was considerably higher than that in the F-TPS group, which also coincided with the LPS content in the blood of mice in both groups. Lactobacillaceae, a common probiotic, was found to up-regulate ileal antimicrobial peptide levels in the intestine and reduce pro-inflammatory cytokines in the circulatory system as well as serum cholesterol levels.^[28] R-TPS and F-TPS promoted an increase of Lactobacillaceae in HFD-fed mice, suggesting that Lactobacillaceae may be involved in the process of anti-obesity effects of R-TPS and F-TPS. Notably, the relative abundance of Lactobacillaceae in the F-TPS group was significantly higher than that in the R-TPS group, which may also explain the lower levels of lipids and inflammation in F-TPS group compared with R-TPS group.

Increasing studies have indicated that *Akkermansia* is a probiotic with great potential. *Akkermansia* may reduce dietary obesity by decreasing the efficiency of food energy and ameliorate obesity by decreasing the

absorption of sugar, and *Akkermansia* also increases energy excretion from the feces and promotes cell renewal in the intestinal wall^[5, 25, 29]. The administration of R-TPS and F-TPS promoted the increase of *Akkermansia* in mice fed with HFD, suggesting that R-TPS and F-TPS may inhibit obesity by decreasing the body's utilization of energy, and may also promote the renewal of intestinal surface cells to protect the integrity of the intestinal barrier. Our experimental results showed that the relative abundance of intestinal *Akkermansia* was significantly higher in the R-TPS group of mice compared with the F-TPS group, and the differences in the expression of genes related to intestinal barrier integrity between the R-TPS and F-TPS groups may be attributed to this. *Muribaculum* has been shown to participate in the bile acid conjugation, reducing hepatic cholesterol production and enhancing overall host health^[30, 31]. R-TPS and F-TPS administration increased the abundance of *Muribaculum* in HFD-fed mice, indicating that the lipid-lowering mechanism of R-TPS and F-TPS may be related to the promotion of *Muribaculum*.

This suggests that the mechanism of lipid-lowering activity of R-TPS and F-TPS may be related to promoting the increase of *Muribaculum*. In addition, R-TPS and F-TPS administration markedly decreased the abundance of *Coriobacteriaceae_UCG-002* and *Ruminococcaceae_UCG-014*, and increased the abundance of *Lachnospiraceae_NK4A136_group* and *Prevotellaceae_UCG-001*. *Coriobacteriaceae_UCG-002* promotes intestinal inflammation and epithelial permeability.^[16, 32, 33] *Lachnospiraceae_NK4A136_group* and *Prevotellaceae_UCG-001* are reported to be producers of SCFA, which may exert weight loss activity by enhancing intestinal barrier function and increasing energy expenditure.^[34] Moreover, Bacteroidetes could alter commensal flora and energy metabolism, which facilitate the modulation of insulin resistance and reduced fat accumulation in obese individuals^[2]. Together, these results suggest that R-TPS and F-TPS intervention attenuated HFD-induced gut microbiota dysbiosis, and R-TPS and F-TPS showed large differences in regulating gut flora, mainly focusing on Firmicutes, Bacteroidetes, and Verrucomicrobia, which may also account for the differences in weight loss effects between R-TPS and F-TPS.

The distinct modulatory effects of R-TPS and F-TPS on gut microbiota are likely attributable to their structural differences. F-TPS, characterized by a higher molecular weight and higher galactose and arabinose content, may resist rapid fermentation in the proximal colon, thereby reaching the distal colon to promote specific fermenters like Muribaculaceae, Lachnospiraceae, and Lactobacillaceae. In contrast, R-TPS, which is rich in uronic acid, provides a specific substrate niche that favors the growth of *Akkermansia*. These structural variations dictate the bioavailability of the polysaccharides to different microbial communities, ultimately leading to the divergent metabolic outcomes observed.

To further investigate whether the weight loss effects of R-TPS and F-TPS were reliant on changes in intestinal flora, the intestinal flora of R-TPS- and F-TPS-treated donor mice was transplanted into HFD-treated recipients by FMT experiments. FMT is not only an important tool for the treatment of certain intestinal flora-related diseases, but also an effective way to investigate the role of specific intestinal flora regulated by functional components in the therapeutic effects.^[3, 35] At present, the activities of many natural products have been proved by FMT experiments to be related to the regulatory effects of intestinal flora, such as the anti-

obesity effects of Fu brick tea, the regulatory effects of aged oolong tea and raw Pu-erh tea on mice with dextran sulfate sodium-induced colitis, etc.^[15, 36, 37]

We found that the gut microbiota of R-TPS- and F-TPS-treated mice effectively reduced HFD-induced obesity and obesity-related features including insulin resistance, abnormal lipid metabolism, and chronic inflammation, and protected the integrity of the intestinal barrier and promoted fat browning, which was similar to that of the effects of R-TPS- and F-TPS-treated mice. In addition, the gut microbiota of R-TPS and F-TPS-treated recipient mice also attenuated HFD-induced gut microbiota dysbiosis, and the gut microbiota of the recipient mice was similar to that of the corresponding donor mice to some extent. Moreover, differences between R-TPS and F-TPS in regulating the abundance of Firmicutes, Bacteroidetes, and Verrucomicrobia were also presented in the intestinal flora of recipient mice.

Despite these promising findings, there are several limitations in the current study that warrant further investigation. First, although the intervention dose (300 mg/kg/d) was rationally selected based on prior literature and preliminary efficacy screening, only a single dose was used in this study, future studies should include a dose-response analysis to determine the optimal therapeutic window and safety profile. Second, while FMT experiments confirmed the crucial role of gut microbiota, we cannot completely rule out the potential influence of residual polysaccharides in the fecal slurry, although the preparation process (centrifugation and washing) aimed to minimize this. Future work using co-housing experiments or comparing viable vs. heat-killed microbiota transplantation could further validate the microbiota-dependent mechanism. Additionally, because microbial samples were only sequenced at the 14-week endpoint, we were unable to dynamically track the colonization stability over the entire transplantation period. Future studies should incorporate longitudinal fecal sampling to monitor the temporal dynamics of the FMT efficiency. Third, this study focused on the total polysaccharide fractions to evaluate their overall bioactivity. As polysaccharides are complex mixtures, the specific structure-activity relationships (SAR) remain to be fully elucidated. Subsequent studies will involve the bioactivity-guided fractionation of R-TPS and F-TPS to identify the specific active components responsible for the differential modulation of gut microbiota. Finally, while we observed significant changes in lipid metabolism-related proteins (e.g., PPARs, SREBP-1c) and inflammatory markers (e.g., NF- κ B pathway), further research is needed to explore the upstream signaling kinases and detailed molecular events governing these pathways. Furthermore, while we observed significant associations between specific microbial taxa (e.g., Muribaculaceae or *Akkermansia*) and metabolic improvements based on Spearman correlations, these results alone cannot confirm causality. Further targeted validations, such as mono-colonization experiments or the use of specific microbial knockout models, are essential to definitively establish the precise roles of these key taxa.

In summary, this study demonstrated that both R-TPS and F-TPS effectively reduced HFD-induced obesity in mice by modulating the gut microbiota, though their mechanisms of weight loss differed. F-TPS showed greater efficacy in regulating glucose homeostasis, promoting lipolysis, and reducing serum LPS and inflammatory factors, while R-TPS was more effective in inhibiting adipogenesis. These differences may be

attributed to their distinct effects on the abundance of Firmicutes, Bacteroidetes, and Verrucomicrobia in the gut microbiota (Fig. 7). In conclusion, while both polysaccharides are effective, their mechanisms are distinct. R-TPS is more effective for managing adipogenesis and enhancing intestinal barrier function via the enrichment of Akkermansia, making it suitable for obesity phenotypes characterized by gut barrier defects. In contrast, F-TPS is superior for regulating glucose homeostasis and systemic inflammation via Muribaculaceae, Lachnospiraceae, and Lactobacillaceae, suggesting its potential for obesity accompanied by insulin resistance. These findings support the development of R-TPS and F-TPS as precision functional foods tailored to specific metabolic phenotypes.

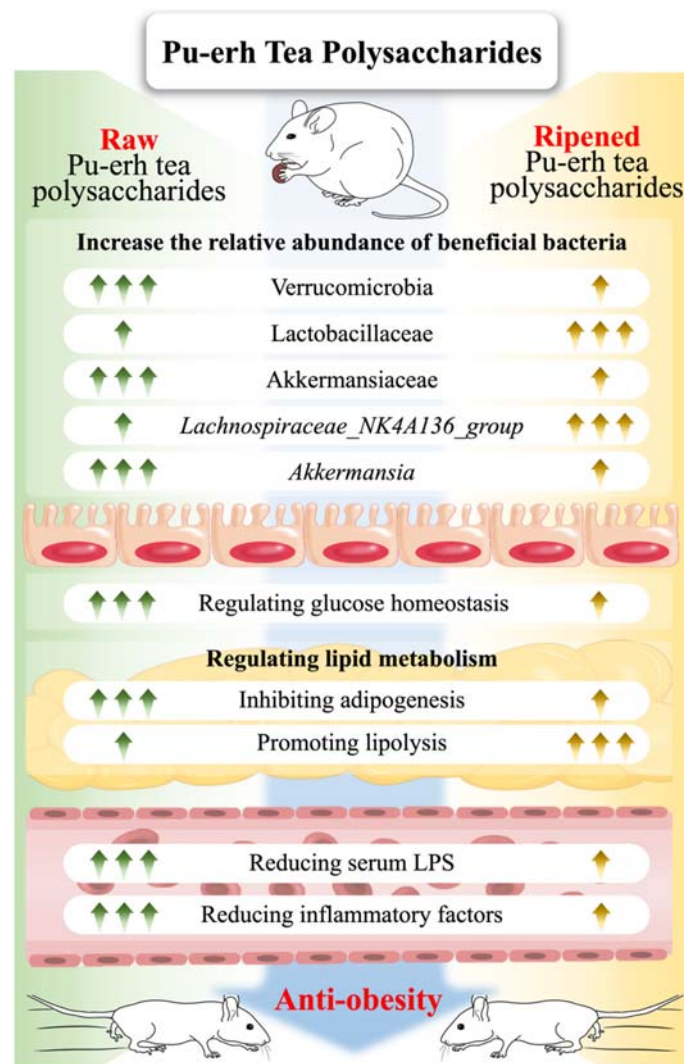


Fig. 7 Mechanistic diagram of R-TPS and F-TPS treatment on obesity.

Declaration of competing interest

The authors declare no competing financial interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by the National Natural Science Foundation of China (81703390) and the Major Project of Science and Technology of Xiamen, grant number 3502Z20226032.

Availability of data and materials

Data is provided within the manuscript and supplementary information files.

Acknowledgements

We would like to thank Instrumental Analysis Center of Huaqiao University for monosaccharide compositions and HPGPC analysis.

References

- [1] W.H.O. (WHO), Obesity and overweight, <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>. (2024),
- [2] T. Zhao, Q. Chen, Z. Chen, et al., Anti-obesity effects of mulberry leaf extracts on female high-fat diet-induced obesity: Modulation of white adipose tissue, gut microbiota, and metabolic markers, *Food Research International*. 177 (2024) 113875-113889. <https://doi.org/10.1016/j.foodres.2023.113875>.
- [3] P. Wang, X. Yang, L. Zhang, et al., Tlr9 deficiency in B cells leads to obesity by promoting inflammation and gut dysbiosis, *Nature Communications*. 15 (2024) 4232-4247. <https://doi.org/10.1038/s41467-024-48611-8>.
- [4] J. Xu, Y. Dai, Y. Bai, et al., Enzymatic pretreatment of *Panax notoginseng* leaves alleviates obesity and modulates the gut microbiota in high-fat diet-fed mice, *Journal of Functional Foods*. 116 (2024) 106155-106167. <https://doi.org/10.1016/j.jff.2024.106155>.
- [5] Q. Zhang, R. Zeng, J. Tang, et al., The “crosstalk” between microbiota and metabolomic profile in high-fat-diet-induced obese mice supplemented with *Bletilla striata* polysaccharides and composite polysaccharides, *International Journal of Biological Macromolecules*. 262 (2024) 130018-130030. <https://doi.org/10.1016/j.ijbiomac.2024.130018>.
- [6] Y. Hou, Z. Zhang, Y. Cui, et al., Pu-erh tea and theabrownin ameliorate metabolic syndrome in mice via potential microbiota-gut-liver-brain interactions, *Food Research International*. 162 (2022) 112176-112188. <https://doi.org/10.1016/j.foodres.2022.112176>.
- [7] X. Pang, W. Yu, C. Cao, et al., Comparison of Potent Odorants in Raw and Ripened Pu-Erh Tea Infusions Based on Odor Activity Value Calculation and Multivariate Analysis: Understanding the Role of Pile Fermentation, *Journal of Agriculture Food and Chemistry*. 67 (2019) 13139-13149. <https://doi.org/10.1021/acs.jafc.9b05321>.
- [8] Y. Xia, D. Tan, R. Akbary, et al., Aqueous raw and ripe Pu-erh tea extracts alleviate obesity and alter cecal microbiota composition and function in diet-induced obese rats, *Applied Microbiology and Biotechnology*. 103 (2019) 1823-1835. <https://doi.org/10.1007/s00253-018-09581-2>.
- [9] S.-H. Tu, M.-Y. Chen, L.-C. Chen, et al., Pu-erh Tea Extract Attenuates Nicotine-Induced Foam Cell Formation in Primary Cultured Monocytes: An in Vitro Mechanistic Study, *Journal of Agricultural and Food Chemistry*. 64 (2016) 3186-3195. <https://doi.org/10.1021/acs.jafc.6b00624>.
- [10] X. Lu, J. Liu, N. Zhang, et al., Ripened Pu-erh Tea Extract Protects Mice from Obesity by Modulating Gut Microbiota Composition, *Journal of Agriculture Food and Chemistry*. 67 (2019) 6978-6994. <https://doi.org/10.1021/acs.jafc.8b04909>.
- [11] J. Ye, Y. Zhao, X. Chen, et al., Pu-erh tea ameliorates obesity and modulates gut microbiota in high fat diet fed mice, *Food Research International*. 144 (2021) 110360-110372. <https://doi.org/10.1016/j.foodres.2021.110360>.
- [12] G. Chen, R. Chen, D. Chen, et al., Tea polysaccharides as potential therapeutic options for metabolic diseases, *Journal of Agriculture Food and Chemistry*. 67 (2018) 5350-5360. <https://doi.org/10.1021/acs.jafc.8b05338>.

- [13] Y. Di, Y. Song, K. Xu, et al., Chicoric Acid Alleviates Colitis via Targeting the Gut Microbiota Accompanied by Maintaining Intestinal Barrier Integrity and Inhibiting Inflammatory Responses, *Journal of Agriculture Food and Chemistry*. 72 (2024) 6276-6288. <https://doi.org/10.1021/acs.jafc.3c08363>.
- [14] H. Du, Y. Han, G. Ma, et al., Dietary intake of whole king oyster mushroom (*Pleurotus eryngii*) attenuated obesity via ameliorating lipid metabolism and alleviating gut microbiota dysbiosis, *Food Research International*. 184 (2024) 114228-114237. <https://doi.org/10.1016/j.foodres.2024.114228>.
- [15] M. Zhu, J. Ouyang, F. Zhou, et al., Polysaccharides from Fu brick tea ameliorate obesity by modulating gut microbiota and gut microbiota-related short chain fatty acid and amino acid metabolism, *The Journal of Nutritional Biochemistry*. 118 (2023) 109356-109366. <https://doi.org/10.1016/j.jnutbio.2023.109356>.
- [16] G. Zhao, J. Teng, R. Dong, et al., Alleviating effects and mechanisms of action of large-leaf yellow tea drinking on diabetes and diabetic nephropathy in mice, *Food Science and Human Wellness*. 12 (2023) 1660-1673. <https://doi.org/10.1016/j.fshw.2023.02.023>.
- [17] Y. Liu, S. Lei, R. Hou, et al., Tea polysaccharides from Taiping Houkui may serve as a potential candidate for regulation of lipid metabolism: Roles of gut microbiota and metabolite in vitro, *Journal of Functional Foods*. 102 (2023) 105469-105478. <https://doi.org/10.1016/j.jff.2023.105469>.
- [18] W.-w. Fang, K.-f. Wang, F. Zhou, et al., Oolong tea of different years protects high-fat diet-fed mice against obesity by regulating lipid metabolism and modulating the gut microbiota, *Food & Function*. 14 (2023) 2668-2683. <https://doi.org/10.1039/d2fo03577d>.
- [19] J. Wang, J. Hao, D. Miao, et al., Compound chenpi tea consumption reduces obesity-related metabolic disorders by modulating gut microbiota and serum metabolites in mice, *Journal of the Science of Food and Agriculture*. 104 (2024) 431-442. <https://doi.org/10.1002/jsfa.12940>.
- [20] A. Perino, H. Demagny, K. Schoonjans, A microbial-derived succinylated bile acid to safeguard liver health, *Cell*. 187 (2024) 2687-2689. <https://doi.org/10.1016/j.cell.2024.04.020>.
- [21] P. Paone, D. Latousakis, R. Terrasi, et al., Human milk oligosaccharide 2'-fucosyllactose protects against high-fat diet-induced obesity by changing intestinal mucus production, composition and degradation linked to changes in gut microbiota and faecal proteome profiles in mice, *Gut*. 73 (2024) 1632-1649. <https://doi.org/10.1136/gutjnl-2023-330301>.
- [22] H. Zhou, X. Wang, Z. She, et al., Combining bioinformatics and multiomics strategies to investigate the key microbiota and active components of Liupao tea ameliorating hyperlipidemia, *Journal of Ethnopharmacology*. 333 (2024) 118438-118454. <https://doi.org/10.1016/j.jep.2024.118438>.
- [23] D. Yang, J. Shen, C. Tang, et al., Prevention of high-fat-diet-induced obesity in mice by soluble dietary fiber from fermented and unfermented millet bran, *Food Research International*. 179 (2024) 113974-113987. <https://doi.org/10.1016/j.foodres.2024.113974>.
- [24] L. Yi-Fan, L. Nan, Z. Bing, et al., Flavonoid-Rich mulberry leaf extract modulate lipid metabolism, antioxidant capacity, *Food Medicine and Health*. 1 (2024) 9420016-9420029. <https://doi.org/10.26599/FMH.2024.9420016>.
- [25] X. Zhu, L. Zhao, L. Lei, et al., Fecal microbiota transplantation ameliorates abdominal obesity through inhibiting microbiota-mediated intestinal barrier damage and inflammation in mice, *Microbiological Research*. 282 (2024) 127654-127667. <https://doi.org/10.1016/j.micres.2024.127654>.
- [26] H. Su, W.J. Wang, G.D. Zheng, et al., The anti-obesity and gut microbiota modulating effects of taxifolin in C57BL/6J mice fed with a high-fat diet, *Journal of the Science of Food and Agriculture*. (2021) 11496-11506. <https://doi.org/10.1002/jsfa.11496>.
- [27] R. Duan, X. Guan, K. Huang, et al., Flavonoids from Whole-Grain Oat Alleviated High-Fat Diet-Induced Hyperlipidemia via Regulating Bile Acid Metabolism and Gut Microbiota in Mice, *Journal of Agriculture Food and Chemistry*. 69 (2021) 7629-7640. <https://doi.org/10.1021/acs.jafc.1c01813>.
- [28] Y. Peng, W. Dong, G. Chen, et al., Anthocyanins from *Lycium ruthenicum* Murray Ameliorated High-Fructose Diet-Induced Neuroinflammation through the Promotion of the Integrity of the Intestinal Barrier and the Proliferation of *Lactobacillus*, *Journal of Agriculture Food and Chemistry*. 71 (2023) 2864-2882. <https://doi.org/10.1021/acs.jafc.2c06713>.

-
- [29] B. Wei, Z. Peng, W. Zheng, et al., Probiotic-fermented tomato alleviates high-fat diet-induced obesity in mice: Insights from microbiome and metabolomics, *Food Chemistry*. 436 (2024) 137719-137730. <https://doi.org/10.1016/j.foodchem.2023.137719>.
- [30] Y. Song, H. Shen, T. Liu, et al., Effects of three different mannans on obesity and gut microbiota in high-fat diet-fed C57BL/6J mice, *Food & Function*. 12 (2021) 4606-4620. <https://doi.org/10.1039/d0fo03331f>.
- [31] M.H. Do, H.B. Lee, E. Lee, et al., The Effects of Gelatinized Wheat Starch and High Salt Diet on Gut Microbiota and Metabolic Disorder, *Nutrients*. 12 (2020) 1-14. <https://doi.org/10.3390/nu12020301>.
- [32] Z. Li, R. Sang, G. Feng, et al., Microbiological and metabolic pathways analysing the mechanisms of alfalfa polysaccharide and sulfated alfalfa polysaccharide in alleviating obesity, *International Journal of Biological Macromolecules*. 263 (2024) 130334-130353. <https://doi.org/10.1016/j.ijbiomac.2024.130334>.
- [33] Z. Yu, D. Li, H. Sun, Herba Origani alleviated DSS-induced ulcerative colitis in mice through remodeling gut microbiota to regulate bile acid and short-chain fatty acid metabolisms, *Biomedicine & Pharmacotherapy*. 161 (2023) 114409-114418. <https://doi.org/10.1016/j.biopha.2023.114409>.
- [34] M. Peng, C. Wang, Z. Gao, et al., Citrus aurantium L. 'Daidai' physiological premature fruit drop relieves obesity in high-fat-diet-fed mice via modulating lipid metabolism and gut microbiota, *Food Bioscience*. 61 (2024) 104713-104725. <https://doi.org/10.1016/j.fbio.2024.104713>.
- [35] H. Li, L. Zhang, J. Li, et al., Resistant starch intake facilitates weight loss in humans by reshaping the gut microbiota, *Nature Metabolism*. 6 (2024) 578-597. <https://doi.org/10.1038/s42255-024-00988-y>.
- [36] J. Wu, X. Deng, Y. Sun, et al., Aged oolong tea alleviates dextran sulfate sodium-induced colitis in mice by modulating the gut microbiota and its metabolites, *Food Chemistry X*. 21 (2024) 101102-101115. <https://doi.org/10.1016/j.fochx.2023.101102>.
- [37] S. Zhou, J. Yang, Y. Pan, et al., Pu' er raw tea extract alleviates DSS-induced colitis in mice by restoring intestinal barrier function and maintaining gut microbiota homeostasis, *Food Bioscience*. 53 (2023) 102750-102760. <https://doi.org/10.1016/j.fbio.2023.102750>.